

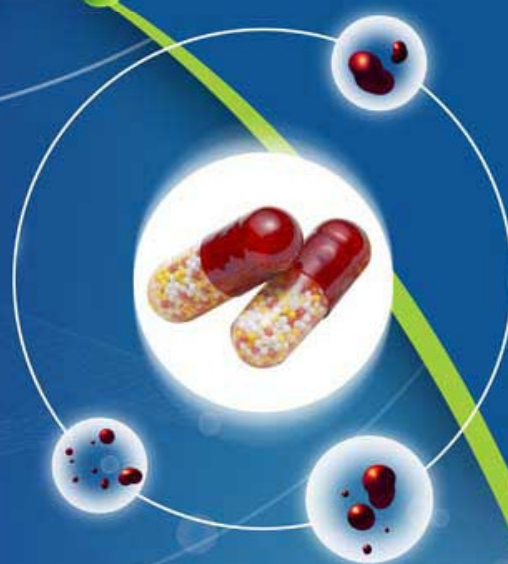


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## Research Article

## METHOD DEVELOPMENT & SIMULTANEOUS ESTIMATION OF METFORMIN HYDROCHLORIDE AND REPAGLINIDE IN TABLET DOSAGE FORM

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### ABSTRACT

Development and Validation of RP-HPLC Method for Simultaneous Estimation of MET and RPG in Tablet Dosage Form was performed by using LC system consists of pump (Shimadzu LC 10AT VP) with universal loop injector (Rheodyne 7725 i) of injection capacity 20  $\mu$ L. Detector consists of Photodiode Array Detector (PDA) SPD-10 AVP UV-Visible detector, for separation column used was Chromatopak C18 ( $5\mu\text{m} \times 250\text{mm} \times 4.6\text{mm i.d.}$ ). For quantitative estimation of MET and RPG, mobile phase used was acetonitrile : water in the volume of 90 : 20 v/v with the flow rate of 1.0 ml/min was confirmed by considering the resolution between both the peaks. Detection wavelength 223 nm was selected as both the analyte absorbed well and at this particular wavelength neither any interference from the mobile phase nor any baseline disturbance appeared. Validation reveals the method is specific, rapid, accurate, precise, reliable and reproducible. The high recovery and low coefficients of variation confirm the suitability of the method of simultaneous analysis of the two drugs in tablets.

**Key words:** HPLC, Metformin, Repaglinide

### INTRODUCTION

Metformin Hydrochloride 1-(diaminomethylidene)-3,3-dimethylguanidine Hydrochloride is a Biguanides antidiabetic which is white crystalline powder, hygroscopic, odorless and bitter in taste. Repaglinide S-2-Ethoxy-4-[2-[[methyl-1-[2-[(1-piperidinyl) Phenyl]-butyl] amino]-2-oxoethyl] benzoic acid is a Meglitinide antidiabetic which is white to off white in color.

A literature survey reveals that MET and RPG have been reported separately by HPLC and UV Spectrophotometry. Since no chromatographic method has been developed yet for the combination of these two drugs and thus this combination was selected in order to develop a chromatographic method. [1]

### Experiment

Pure sample of MET and RPG were obtained from was obtained from Modern Laboratories, Indore, India. and Torrent pharma Ltd Gujarat, India respectively. The tablet dosage form, Prandimet, manufactured by Novo Nordisk Ltd, Bangalore (Label claim: 500 mg metformin and 2 mg repaglinide) was procured locally. All chemicals

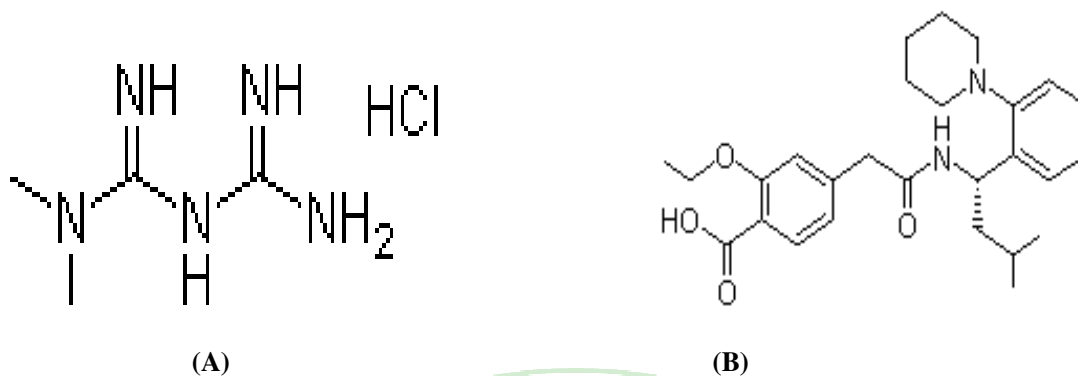
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and reagents used were of HPLC grade and purchased from Spectrochem, Mumbai, India.



**Fig.1. The chemical structure of MET (A) and RPG (B)**

#### **Preparation of Standard and Sample Solutions**

MET and RPG (10mg each) were weighed accurately and separately transferred to 100ml volumetric flasks. Two drugs were dissolved in acetonitrile to prepare standard stock solution containing 100 $\mu$ /ml.

For analysis of tablet dosage form, twenty tablets were weighed individually and their average weight was determined. The tablets were crushed to a fine powder and powder equivalent to the weight of one tablet was transferred to a 100ml volumetric flask and dissolved in 30ml acetonitrile. The solution was shaken vigorously for 15min and filtered through Whatman #41 filter paper, and the residue was washed with solvent. The solution was then dissolved to a volume with same solvent.

#### **Chromatography**

The LC<sup>2</sup> system consists of pump (Shimadzu LC 10AT VP) with universal loop injector (Rheodyne 7725 i) of injection capacity 20  $\mu$ L. Detector consists of photodiode array detector (PDA) SPD-10 AVP UV-Visible detector, for separation column used was Chromatopak C<sub>18</sub> (5 $\mu$ m $\times$ 250mm $\times$ 4.6mm i.d). The equipment was controlled by a PC workstation equipped with software CLASS M 10-VP software (Shimadzu, Kyoto, Japan). The volume capacity of the reservoir was greater than 500ml. The mobile phase velocity was within 1-2 ml/min.

The mobile phase was a 90:20(v/v) mixture of acetonitrile and water at a flow rate of 1mL min<sup>-1</sup>. The run time was 10min. Before analysis both mobile phase and sample solution were degassed by sonication and filtered through 0.2- $\mu$ m filter paper. The analytes were monitored at 223nm. The two components were identified by comparison of retention time obtained from sample and standard solutions. The work was performed in an air-conditioned room maintained at 25 $\pm$ 2 $^{\circ}$ C.

#### **Construction of calibration plots**

From the standard stock solutions of two drugs MET and RPG, different dilutions were prepared for each drug and chromatographed and the peak area was measured. From the calibration it was found that response to MET and RPG was linear function of concentration in the range 5-40 $\mu$ g mL<sup>-1</sup> and 2-35  $\mu$ g mL<sup>-1</sup>. Unknown assay samples were quantified by reference to this calibration plots.

#### **Assay of Tablet Formulation**

Before assay of tablet formulations six replicates of the required dilution were prepared from the tablet stock solution and sonicated. The solutions (20 $\mu$ L) were then injected for quantitative analysis. The amount of MET and RPG per tablet were calculated by extrapolating the peak area from the calibration curve. [2-6]



**Table 1: RESULTS FROM ASSAY OF THE TABLET FORMULATION**

Drug	Label claim (mg/tablet) n=6	Amount Found in mg	Drug Concentration (%)
MET	500	498.48	99.696
RPG	2	1.987	99.35

MET: Metformin, RPG: Repaglinide

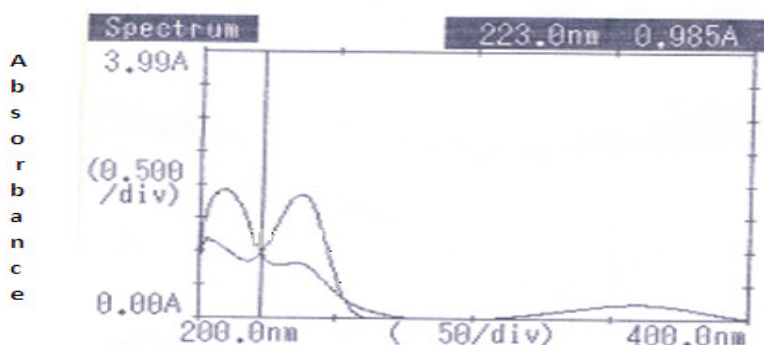
### Validation

The method was validated for linearity, accuracy, precision, reapiability, selectivity, and specificity. Accuracy was studied by measurement of recovery at three different level 80, 100, 120% of the amount expected in the formulation, in accordance with ICH guidelines<sup>3</sup>. Precision measured at both intra-day and inter-day. In the intraday study the concentration of two drugs were calculated three times on the same day at interval of an hour. In the inter-day study the concentration of two drug were measured on three different days. The selectivity and specificity of the method will validated by injecting solution containing two drugs; two sharp peaks obtain for two drugs. The limit of detection and quantitation of the method were studied to detect the lowest amount of analyte and quantitative determination of analyte in a sample, respectively.

### HPLC Method Development and Optimization

Column chemistry, solvent type, solvent strength, detection wavelength, and flow rate were varied to determine the chromatographic conditions giving the best separation. The mobile conditions were optimized so the tablet components free from interference from the solvent and from the excipient. Other criterias, for example time required

for analysis, appropriate k range for eluted peaks, assay sensitivity, solvent noise, and use of same solvent system for extraction of the drug from formulation matrices during drug analysis were also considered. After trying column containing different stationary phase, the final choice giving satisfactory resolution and run time was the 25cm×4.6mm i.d., 5µm particle, Chromatopack C18 reversed phase column. A series of aqueous mobile phase in combination with different volume fraction of acetonitrile as modifier were also tested. The best result was obtained with acetonitrile: water in the ratio of 90:20 (v/v). The flow rate was determined by testing the effect of different flow rates on peak area and resolution; 1.0mL min<sup>-1</sup> was found to be optimum. The experiment was performed at ambient temperature. To determine the appropriate wavelength for simultaneous determination of MET and RPG, solution of these compound in acetonitrile by UV-visible spectrophotometry (Shimadzu 1700) in the range of 200-400nm. From the overlain spectra, suitable wavelength considered for monitoring the drugs were 223nm. Solution of each substance in acetonitrile were also injected directly for HPLC analysis and the responses (peak) were recorded at 223nm. It was observed there was no interference from the mobile phase or baseline disturbance and all the analyte absorbed well at 223nm. [7-9]

**FIG 1: Overlain spectra**

Under the optimum chromatographic condition, the retention time obtained for MET and RPG were 2.72 and 6.13 min respectively (fig-3). Resolution ( $R_s$ ) between MET and RPG was 17.03 respectively. Capacity factor, Tailing factor, no of theoretical plates are reported in Table-2.

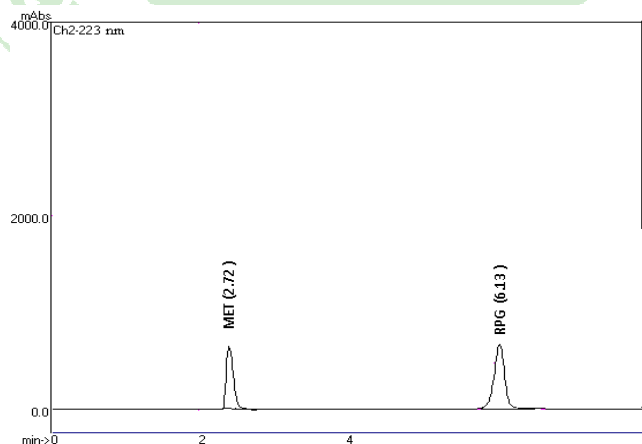
**Table 2: Result from system suitability study for standard**

<i>Property</i>	<i>MET</i>	<i>RPG</i>
$R^t$	2.72	6.13
$T_f$	1.20	1.16
$K'$	0.30	0.05
$N$	6859	8819
$R_s$	-	17.03

$R_t$  : Retention time,  $T_f$  : Tailing factor,  $K'$ : Capacity factor,  $N$  : Theoretical plate number,  $R_s$ : Resolution

**Table 3: Result from system suitability study for sample**

<i>Property</i>	<i>MET</i>	<i>RPG</i>
$R^t$	2.72	6.13
$T_f$	1.13	1.15
$K'$	0.29	0.03
$N$	6752	8655
$R_s$	-	16.11



**Fig-3. Chromatogram obtained from MET and RPG in sample solution, with retention time.**

The values obtained for  $k$  and  $R_s$  ( $1 < 10$ ,  $R_s > 2$ ) shows these chromatographic condition are appropriate for separation and separation and quantification of two compounds. The number of plates ( $N$ ) is a measure of column efficiency; which shows the high separation efficiency of the column used.

#### Validation of the Method

The method was validated for linearity, accuracy, precision, repeatability, selectivity, and specificity. All validation studies were performed by replicate injection of sample and standard solutions.

#### Linearity

For each drug, appropriate dilutions of standard stock solutions were assayed as per the developed methods. MET has linearity between 5-40  $\mu\text{g/ml}$  and linearity of RPG has range between 2-35  $\mu\text{g/ml}$  the linear regression equation for two drugs was; [10-11]

$$\text{Metformin} \quad y = 54750x + 25034 \quad (n = 5, r^2 = 0.997)$$

$$\text{Repaglinide} \quad y = 78595x + 17972 \quad (n = 5, r^2 = 0.995)$$

Where  $y$ : peak area and  $x$ : concentration.

**Table 4: Result from recovery study**

Metformin	Amount taken ( $\mu\text{g/mL}$ )	Amount added		Recovery(%, $\pm$ SD)	%COV
		%	$\mu\text{g/ml}$		
1.	500	80	400	0.0193	0.0003
2.		100	500	0.0181	0.0003
3.		120	600	0.0349	0.0012

**Table 5: Result from recovery study**

Repaglinide	Amount taken ( $\mu\text{g/mL}$ )	Amount added		Recovery(%, $\pm$ SD)	%COV
		%	$\mu\text{g/ml}$		
1.	2	80	1	0.767	0.5896
2.		100	2	0.664	0.4418
3.		120	3	0.940	0.8848

S.D., standard deviation; COV, coefficient of variance.

#### Precision, LOD and LOQ

Precision was studied both intra-day and inter-day. Six replicate sample solution were prepared from the stock solution. For study of intra-day precision the concentration of two drugs were measured three times on the same day at interval of 1hr. In the

inter-day study the drug concentration were measured on three different days. The limits of detection and quantitation, LOD and LOQ, were calculated by use of equation  $\text{LOD} = 3.3\sigma/S$  and  $\text{LOQ} = 10\sigma/S$ , where  $\sigma$  is the standard deviation of the blank and  $S$  is the slope of the calibration plot.

Table 6: Result from Precision and LOD and LOQ

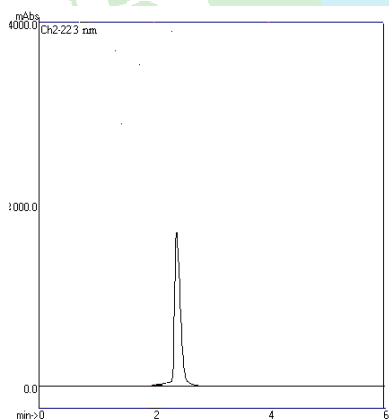
<i>Parameters</i>	<i>Metformin</i>	<i>Repaglinide</i>
<b>LOD</b>	0.5278 $\mu$ g/ml	0.105 $\mu$ g/ml
<b>LOQ</b>	1.599 $\mu$ g/ml	0.319 $\mu$ g/ml
<b>Intra-day precision study</b>		
<b>%COV</b>	0.0540	0.3227
<b>S.D</b>	0.2324	0.7951
<b>Inter-day precision study</b>		
<b>%COV</b>	0.005	0.3845
<b>S.D</b>	0.0907	0.6201

COV, coefficient of variance; LOD, limit of detection; LOQ, limit of quantitation

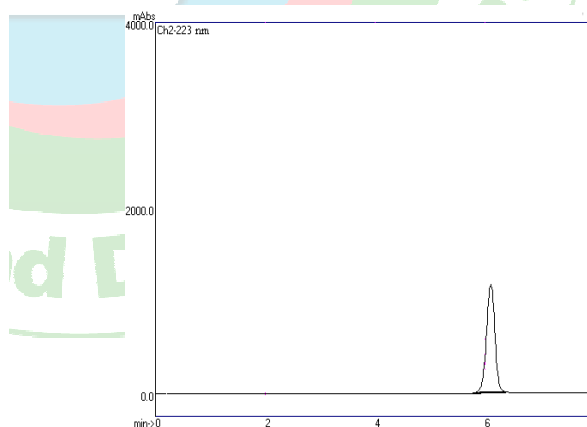
#### Selectivity and specificity

Selectivity of the developed method was conformed as the resolution between the chromatograms of MET and RPG was 17.03. This suggests that under the proposed chromatographic conditions MET and RPG were completely separated from each other. Which indicates the method is selective for simultaneous estimation.

The specificity of the method was assessed by comparing chromatogram obtained from drug standards (Fig.4) with that obtained from tablet solutions. The retention time of the drug standards and the drugs from the tablet solutions were same, so the method was specific. The method was also specific and selective because there was no interference from excipients in the tablets.



(A)



(B)

Fig-4 Chromatogram obtained from MET(A) with Rt of 2.72(A), PG(B) with Rt of 6.13

## CONCLUSION

The developed methods for simultaneous estimation of MET and RPG in tablet dosage form using RP- HPLC was found statistically significant as the statistical results of validation study were within the acceptance range. This reveals that the developed method was simple, accurate, precise, selective and specific for simultaneous estimation of both the drugs from commercially available dosage form.

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